UNIVERSITAS
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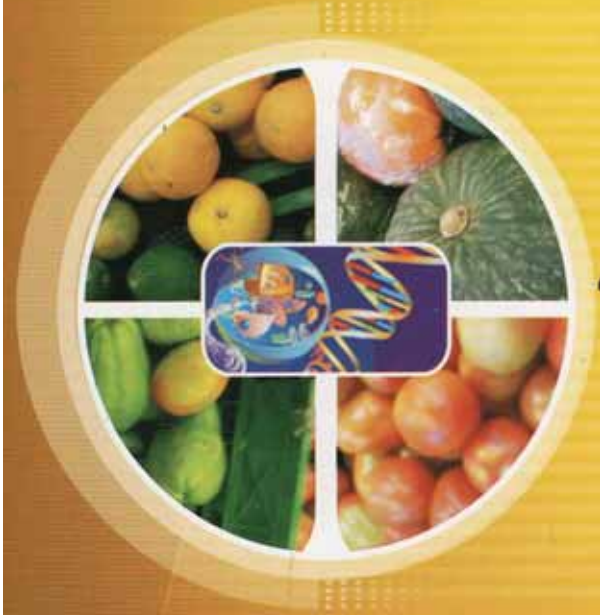

1st International Seminar on
**"Natural Resources Biotechnology: From Local to Global"**

September 8th – 9th 2015
Faculty of Biotechnology
Universitas Atma Jaya Yogyakarta

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Faculty of Biotechnology Universitas Atma Jaya Yogyakarta

# CERTIFICATE

This certificate is awarded to

## Dr. Mufasirin, M.Si., drh.

in recognition of their valuable contribution as

## ORAL PRESENTER

In The 1st International Seminar on
**"Natural Resources Biotechnology: From Local to Global"**
organized by Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta on
September 8-9th, 2015 in Yogyakarta, Indonesia.

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## Welcome Speech
## Chair of the Seminar Committee

Distinguished Guests,
Honorable Speakers,
Ladies and Gentlemen,

It is a great pleasure to welcome all of you to the International Seminar "Natural Resources: From Local to Global". The Faculty of Biotechnology of Universitas Atma Jaya Yogyakarta runs this seminar to commemorate the 50th Anniversary of the Universitas Atma Jaya Anniversary and the 25th Anniversary of the Faculty of Biotechnology. Your presence is your present for the anniversary of our university and faculty as well.

The Anniversary is not the only reason to run this seminar. A greater reason is behind the seminar. Indonesia is rich in biodiversity. It is a challenge for us, as scientist, to maintain the biodiversity and to develop the potential of the biodiversity for the common good. Through this seminar, the scientific research on Indonesian biodiversity can be shared and probably the finding of the new research can inspire us for further exploration. Therefore, the seminars goal is to facilitate the spread of the research on local potential of biodiversity to the global level. Hopefully, it can attract more researchers to explore the wealth of local biodiversity.

The committee invites speakers who are expertise in the research concerning biodiversity. Our invited speakers are Assoc. Prof. Dr. Michael Murkovic from Graz University of Technology Austria (food scientist), Assoc.Prof. Worawidh Wajjwalku from Kasetsart University Bangkok Thailand (Veterinary disease biotechnology), Dr. Kathryn McMahon from Edith Cowan University Australia (Seagrass biotechnology), Prof. Marco Nemesio E. Montano, PhD from University of the Philippines (Seaweed biotechnology), Prof. Jun Kawabata from Hokkaido University Japan (food biochemist), Endang Semiarti, PhD from Universitas Gadjah Mada, Indonesia (Plant biotechnology), Ign. Pramana Yudha, PhD from Universitas Atma Jaya Yogyakarta (Conservation genetics), Dr Machmud Thohari from Technical Team for Environmental Biosafety, Ministry of Enviroment & Forestry Indonesia (Environmental Biosafety), Dr Harvey Glick from Asia Regulatory Policy & Scientific Affairs Monsanto Company (Regulatory Policy & Scientific Affairs Monsanto). It is a good opportunity to learn from the speakers to enhance and to update our knowledge. I hope this seminar is of benefit to all of us.

In conclusion, I wish you a successful seminar and a pleasant stay in Yogyakarta.

With kind regard
Coordinator of conference program

Dr. rer. nat. Yuliana Reni Swasti, S.TP., MP.

**WELCOME SPEECH**
**DEAN**
**FACULTY OF BIOTECHNOLOGY**
**UNIVERSITAS ATMA JAYA YOGYAKARTA**

Distinguished Guests,
Honorable Speakers,
Ladies and Gentlemen,

On behalf of the Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta and the Committee of the International Seminar, I would like to first of all to extend our heart-felt thanks for your presence at this Seminar. This seminar is so significant in a sense that it focuses on natural resources with local content but by utilizing biotechnology they will become global and worldwide products and services as well.

Biotechnology has been developed very rapidly and it is believed to be "a new wave in the economic world". Biotechnology has contributed in all aspects of humans' life, such as food production, health, industry, environment, etc. The role of biotechnology for the betterment of human beings, however, is still need to be improved. Indonesia, with its huge biodiversity, has a potency to develop and applied biotechnology nationwide.

The role of biotechnology has increased rapidly. Many are believed that biotechnology has become an integral part of modern industries with high economic values. On the other hand, it needs to be closely managed in order to avoid its negative impacts. The are some example of negative impacts with relate to biotechnology application, such as intellectual property rights, genetically modified organisms (GMOs), environmental degradations, biodiversity issues, indigenous people knowledge, biosafety, etc.

The Seminar covers topics such as: Functional Foods, Food Biotechnology, Biopharmacy, Health/Medical Biotechnology, Environmental Biotechnology, Legal Aspect of Biotechnology, Bioinformatics, and Social-Economic Aspects of Biotechnology. This Seminar will be presented nine (9) invited speakers with different topics and expertise. There will be some papers and posters to be presented also in this Seminar from some participants from the Philippines and Indonesia.

Henceforth, in commemorating its 50th anniversary Universitas Atma Jaya Yogyakarta (UAJY) and 25th anniversary of Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta (UAJY) on September 2015, it is worthy and appropriate to explore the newest innovations in the field of research and development of biotechnology to be applied in many aspects for the betterment of human beings. The Seminar takes this opportunity to discuss and hopefully find ways to solve problems faced by human beings in the world.

I would like to take this opportunity to express my sincere thanks and gratitude to the Committee and in particular to the honorable speakers. Before closing this remarks, allow me to ask the Rector of Universitas Atma Jaya Yogyakarta to open this Seminar officially.

Finally, this is an opportune time for me to wish you all in the two (2) fruitful days of interesting and beneficial programs and hope you have a pleasant stay in Yogyakarta.

Thank you very much and may God bless us all. Amen.

Yogyakarta, 8 September 2015

Dean

Drs. B. Boy Rahardjo Sidharta, M.Sc

# Brief Contents

# Cloning of cDNA Encoding Membrane Protein of Tachyzoite of *Toxoplasma gondii* In pUC19

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## Abstract

The aim of the research is to clone cDNA encoding membrane protein of Toxoplasma gondii using pUC19 for developing molecular diagnosis and vaccines. Total RNA and messenger RNA were isolated from tachyzoites of Toxoplasma gondii that growing up in Swiss strain mice using PolyAtract®mRNA Isolation Systems (Promega) and synthesis of cDNA using Riboclone®cDNA Synthesis Systems (Promega). Complementary DNA was ligated with *Eco*RI adaptor using Riboclone®*Eco*RIAdaptor Systems (Promega). preparation pUC19 vector, pUC19 ligated with cDNA was transformed in *E. Coli* DH5α. Plasmid recombinant was analyzed using endonuclease restriction enzyme (*Eco*RI and *Hind*III), total protein isolation and immunoblotting. Results of transformation using the pUC19 vector obtained two recombinant clones carrying genes encoding membrane proteins.

## 1. INTRODUCTION

Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii*. The disease is spread all over the world, and is more common in cold climate regions[1]. In acute infections, most are asymptomatic, and that really shows just a slight pain symptoms and is usually not diagnosed[2]. Infection in adults showed no clinical symptoms but sometimes showed lymphadenitis in cervical region and other regions, accompanied by a mild fever[3]. In pregnant women and animals, severe infections can lead to miscarriage or congenital defects in children born[1].Toxoplasmosis can be transmitted from animals to humans, so it requires a good disease control strategies. Among other disease control strategies include knowledge of toxoplasmosis, treatment and vaccination.

The use of the vaccine in endemic areas is one appropriate control measures. Toxoplasmosis can be done to control through vaccination of pets, especially cats as the definitive host, *Toxoplasma*-free cattle feeding and limiting contact between humans and cats. *Toxoplasma gondii* vaccines that have been used in the form of a live vaccine (tachyzoite), the surface protein[4], and nucleic acids[5]. Constraints development of live vaccines is the development of the culture and handling of

tachyzoite relatively insecure and expensive, while the surface protein is use vaccine as an antigen supply is not easy. The use of inactivated vaccine and sub unit vaccine of *Toxoplasma* are one alternative to vaccination, but the obstacles faced are contaminants, cost prohibitive in the provision, as well as toxic effects when administered in large quantities[6].

Diagnosis of toxoplasmosis is often based on the presence of antibodies against *Toxoplasma*. Humans and animals showed seropositive for *Toxoplasma* is not necessarily suffering from toxoplasmosis, although once infected with *Toxoplasma*. Diagnosis is confirmed by finding cysts on the brain or tissue and the presence of *Toxoplasma* circulating in the form of bradyzoite or tachyzoite[7]. Presence of antigens in the body can be detected by Enzyme Linked Immunoassay (ELISA) technique[7], Polymerase Chain Reaction (PCR)[8,9,10], Polymerase Chain Reaction - DNA Enzyme Immunoassay (PCR-DEIA)[11]. PCR diagnostics constraint-DEIA or hybridization techniques tracker is available *Toxoplasma* protein for the manufacture of antibodies and DNA is used as a tracer.

*Toxoplasma gondii* is an obligate intracellular protozoan that infects several types of nucleated cells. Contacts among the receptors on host parasite is an initial entry of the parasite into the host[13]. P30 is a major membrane protein is a essential ligand in *Toxoplasma*[12]. P30 is located on the membrane surface and rhoptry of tachyzoite[14]. The development of recombinant DNA technology allows solving the problem of providing a surface protein that is immunogenic in a large amount, so that the manufacture of the vaccine may be cheaper. Recombinant DNA technology can also provide a solution providing both nucleic acid used as a vaccine or material for hybridization tracers in the diagnosis of disease. pUC19 vector is a plasmid often used in engineering is a *Escherichia coli*. The aim of the research is to clone cDNA encoding membrane protein of *Toxoplasma gondii* using pUC19 for developing molecular diagnosis and vaccines.

## 2. METHODS

### 2.1 Total RNA Isolation

Some $10^{10}$ tachyzoites coupled with 1.5 ml of guanidine thiocyanate and mixed by means of resuspended, then transferred into a sterile homogenizer RNase-free tubes. Homogenization is done with the homogenizer 6 times, each with a speed of 1200 rpm for 30 seconds. Homogenization is done in a cold state in order to avoid damage to RNA. Results of homogenization transferred into RNase-free sterile tube, then added with Na-acetate-tenth of the volume and mixed until blended. The solution was put in ice for 5 minutes and added with phenol chloroform as much. Mixing is done by flipping and turning the tube and incubated on ice for 15 minutes. Aquaeus phase obtained by centrifugation with 4000 rpm on 4$^{o}$C for 25 minutes. Aquaeus phase was transferred into a new sterile RNase-free tubes and added isopropanol (1: 1) and mixed with way of flipping through the tube. The solution was incubated at -20$^{o}$C overnight. The solution was centrifuged at 4000 rpm at 4$^{o}$C temperature for 25 minutes. Obtained pellet was washed with cold 75% ethanol and centrifuged at 4000 rpm at 4$^{o}$C for 25 minutes. The supernatant was discarded and the pellets was dried until completely dry at room temperature. The next pellet resuspended with 250 µl RNase-free water.

## 2.2. mRNA Isolation

1) Annealing probe. Messenger RNA was isolated using PolyAtract®mRNA Isolation System. Two hundred and fifty microliters of total RNA coupled with RNase-free water until the volume becomes 500 µl. The solution was incubated at 65$^{o}$C for 10 minutes. Immediately after the incubation, the solution was added with 3 µl biotinylated oligo (dT) probe and 13 µl 20XSSC. The solution was mixed and incubated at room temperature for 10 minutes.

2) Wash streptavidin-paramagnetic particles (SA-PMPs). Streptavidin-paramagnetic particles (SA-PMPs) resuspended to solution, then captured by placing a magnetic stand for 30 seconds until the SA-PMPs collected on the wall (the solution becomes clear). Streptavidin-paramagnetic particles were washed three times with 0.5x SSC (each wash as much as 0.3 ml) in a manner as was done previously and then resuspended in 0.1 ml of 0.5x SSC.

3) The arrest and washing hybrid mRNA with oligo (dT). The reaction solution annealing inserted into the tube containing the SA-PMPs, then mixed and incubated at room temperature for 10 minutes. Complex SA-PMPs with hybrid mRNA and oligo (dT) captured by placing a magnetic stand until the solution becomes clear. Supernatant was discarded carefully no damaging pellets SA-PMPs. Streptavidin-paramagnetic particles were washed four times with 0.1x SSC (each wash as much as 0.3 ml) as shown above, by placing a magnetic stand until the solution becomes clear.

4) Elution of mRNA. SA-PMPs pellet were resuspended in 0.1 ml RNAse free water, then captured by the magnetic stand. Aquaeus phase is taken and put in a new sterile RNase-free tubes. Washing was repeated by adding 0.15 ml of RNase-free water, then SA-PMPs captured using a magnetic stand. Phase aquaeus taken with caution and put in a new sterile tube which had been washed with distilled DEPC.

5) Precipitation and concentration of mRNA. Two hundred and fifty microliters of mRNA elution process results coupled with 25 µl Na-acetate and 250 µl isopropanol, then blended until smooth and incubated on -20$^{o}$C overnight. The reaction mixture was then centrifuged at 12,000 rpm at 4$^{o}$C for 25 minutes. Pellet resuspended in 1 ml of ethanol; 75% cold, then centrifuged at 12,000 rpm at 4$^{o}$C for 25 minutes. The supernatant was discarded and the pellets were dried at room temperature. Pellets resuspended with 10 µl RNase free water and stored at a temperature -70$^{o}$C. mRNA concentrations seen by spectrophotometer at $OD_{260}$.

## 2.3. cDNA Synthesis

*Single strand cDNA synthesis*. Ten microliters of solution containing 2 µg mRNA, plus 1 µl oligo (dT) primer and 4 RNase-free water. The reaction mixture was incubated at 70$^{o}$C for 5 minutes and centrifuged for 5 seconds at a speed of 12,000 rpm for lowering all solution. The reaction mixture was added with 5 µl single strand 5X buffer, 25 U rRNasin ribonuclease inhibitor, 2.5 µl 40 mM sodium pyrophosphate, 15 U AMV reverse transcriptase RNA and RNA free water to a final volume of 25 µl. The reaction solution was mixed well and incubated at 37$^{o}$C for 60 minutes. After incubation, the solution is stored in ice to be used a double strand synthesis.

*Synthesis of double-stranded cDNA*.Twenty microliters of the reaction mixture on the synthesis of single-stranded, plus an additional 10 µl 10X second strand buffer, 23 U

of DNA polymerase I, 0.8 U RNaseH and nuclease free water to obtain a final volume of 100 µl. The reaction solution was mixed well and incubated at 14°C temperature for 3 hours. The polymerization reaction was stopped by heating at 70°C for 10 minutes. The tube containing the reaction mixture was centrifuged at 12,000 rpm for 5 seconds to bring down all liquid and then taken in the ice. The reaction mixture was further added with 2 U T4 DNA polymerase and incubated at 37°C for 10 minutes. Reaction stopped by adding 10 µl 200 mM EDTA and placed on ice. DNA extraction was done by added phenol; isoamylalcohol chloroform 1: 1. Solution mixed well and centrifuged at 12,000 rpm for 2 minutes at room temperature. Aquaeus phase was transferred into a new tube, then added half times the volume of 7.5 M ammonium acetate and two and a half times the volume of cold absolute ethanol. The solution is mixed with gentle, then incubated at -20°C overnight. After incubation, the reaction mixture was centrifuged at 12,000 rpm at 4°C temperature for 25 minutes. Pellet resuspended with cold 75% alcohol and centrifuged at the same speed. The supernatant was discarded and the pellets were dried at room temperature until totally dry. Pellet resuspended with 10-50 µl TE buffer. DNA concentration was measured with a spectrophotometer at $OD_{260}$.

### 2.4. Addition of EcoRI Adapter in cDNA

The addition of the adapter *Eco*RI use Riboclone®*Eco*RI Adaptor Ligation System. A total of 2.5 µl DNA (100 ng/µl) added 3 µl T4 DNA ligase buffer, 3 µl BSA (1 mg/ml), 1 µl *Eco*R I adapter, 2.5 U T4 DNA ligase and nuclease free water to a final volume of 30 µl. The reaction mixture was mixed well and incubated at 15°C temperature overnight. To stop the reaction, the reaction mixture was incubated at 70°C for 10 minutes.

### 2.5. Reaction of Phosphorylation

Thirty microliters of DNA has been ligated with *Eco*RI adapter were added to 4 µl T4 polynucleotide kinase buffer 10X, 2 µl 0.1 mM ATP, 1 µl polynucleotide T4 kinase 10 U and nuclease free water to obtain a final volume of 40 µl. The reaction mixture was mixed well and incubated at 37°C for 30 minutes. The reaction mixture was extracted with phenol chloroform adding as much and blended well with the manner here in after sacker for 30 seconds at a speed of 12,000 rpm centrifuged for 3 minutes. Aquaeus phase was transferred into a new tube and re extraction to obtain maximum results by adding 20 µl TE buffer. Phase aquaeus then added with a half times volume 7.5 M ammonium acetate and two volumes of cold absolute ethanol. The solution was mixed well and incubated at -20°C overnight. To obtain a pellet, a solution was then centrifuged at 3,000 rpm at 4°C for 25 minutes. The supernatant was discarded and the pellet was washed with cold 75% ethanol. Pellets were dried at room temperature until completely dry and resuspensed with 10 µl TE buffer.

### 2.6. Elimination of Excess Adapters

Sephacryl S-400 which has been thoroughly mixed in a tube is inserted into the column and allowed a few minutes for all solvent buffer down to the bottom. Column tube inserted into the tube and centrifuged at 2500 rpm washing with rotor SW for 5 minutes. Centrifugation is repeated when a column of Sephacryl still not perfectly dry. Column tube is taken and DNA samples inserted into the column using a

micropipette. The addition of DNA samples is done right at the top of the column and in each column maximum volume of 60 µl. Column tube inserted into the collector tube and centrifuged at 2500 rpm for 5 minutes. Supernatant are accommodated in the collector tubes ready for ligation reactions with the vector.The addition of the adapter *Eco*RI use Riboclone®*Eco*R I Adaptor Ligation System. A total of 2.5 µl DNA (100 ng/µl) added 3 µl T4 DNA ligase buffer, 3 µl BSA (1 mg/ml), 1 µl *Eco*R I adapter, 2.5 U T4 DNA ligase and nuclease free water to a final volume of 30 µl. The reaction mixture was mixed well and incubated at 15$^{o}$C temperature overnight. To stop the reaction, the reaction mixture was incubated at 70$^{o}$C for 10 minutes.

### 2.7. Preparation pUC19

*Escherichia coli* colonies containing pUC19 on the plate so as propagated by culturing in 10 ml of LB media which already added with ampicillin, and incubated at 37°C overnight. After the bacteria grew, coupled with 100 ml of new LB media was added ampicillin, and incubated at 37°C for 2-3 hours to obtain the logarithmic growth phase ($OD_{600}$ ranging from 0.3 to 0.6). Bacteria are harvested by means of centrifugation at 3000 rpm at 4°C for 20 minutes. The supernatant was discarded and the pellet resuspended with 4 ml of lysing solution I, and mixed by means of tossing and turning the tube, then incubated on ice for 5 minutes. A mixture of bacteria and then added with 6 ml of lysing solution II, and mixed as the above and incubated on ice for 15 minutes. After incubation, add 4.5 ml of lysing solution III, and mixed in the same manner as before. The reaction mixture was then incubated on ice for 15 minutes, and centrifuged at 3000 rpm at 4°C for 20 minutes. Aquaeus phase was transferred into a new tube and added phenol CIAA 1: 1, mixed well and centrifuged at 4°C for 20 minutes. Aquaeus phase was transferred into a new tube and added voleme Na acetate 0.1x, 2.5x the volume of cold absolute ethanol. The reaction mixture was incubated at -20°C overnight, and centrifuged at 3000 rpm at 4°C for 25 minutes. The supernatant was discarded, and the pellet was washed by means resuspended with 75% ethanol and centrifuged at 3000 rpm at 4°C for 25 minutes. Pellets were dried at room temperature and diluted with 100 mL of TE buffer. Concentration measured with a spectrophotometer at $OD_{260}$[15].

### 2.8. pUC19 digestion with *Eco*RI

Four microliters of pUC19 with a concentration of 1 mg / mL, 2 mL buffer coupled with *Eco*RI, 4 ml *Eco*RI and nuclease free water so that the total volume of the final 20 ml and mixed well. The reaction mixture was then incubated at 37$^{o}$C for 2 hours. Cutting reaction results was electrophoresed in a 1% agarose gel at 100 Volts.

### 2.9. Ligation of cDNA Expression Vector

A total of 1 ml pUC19 (200 ng/ml) plus 2 kinase cDNA mL, 1 ml ATP, 1 ml of 10X ligase buffer and 4 µl nuclease T4DNA free water so that the final total volume 10 ml. The reaction mixture was incubated at 15$^{o}$C for 4 hours. Results of the reaction are stored at -20°C and is ready to be transformed in host cells.

### 2.10. Transformation and Analysis of Transformants Results

*Preparation of competent cells*. A single colony of *E. coli* DH5α was cultured into 5 ml LB medium and incubated overnight at 37°C with agitation speed of 200 rpm. Once the bacteria grow, coupled with 25 ml of new LB and incubated for 2-3 hours until the

$OD_{260}$ obtained between from 0.3 to 0.6A total of 3 ml of media containing the bacteria, then harvested in a way centrifuged at 12,000 rpm for 5 seconds, at room temperature. Pellets were washed with sterile distilled water 5 times, 1.5 ml respectively. Pellet resuspended with 200 mL of distilled water and is ready for the process of transformation.

*Transformation by electroporation technique.*Two hundred microliters of solution containing 5 mL of bacterial cells with a plasmid that has been ligated with cDNA sample, and then mixed well. Electroforating done with Gene PulserTM at 2.5 kV. Control the transformation consists of the positive control (*E. coli* with pUC19) and negative control (*E. coli* without pUC19), ligation control and work control (sterility). If the transformation shown in the tool Gene PulserTM under number 13 or above 16, the transformation process is repeated until around 14. Samples were then added new LB media, and mixed well. Samples were incubated at 37°C for 1 hour. After incubation, the samples were centrifuged at 12,000 rpm for 5 seconds, and the supernatant was discarded. Pellet was resuspended in 200 mL LB media new and ready to be planted on the plate in order.

*Planting on the plate*. Sample results of the transformation process and then grown on LB plates that are already coupled with X-gal, IPTG and ampicillin. Especially for sterility control is not coupled with the substances mentioned above. The number of samples that were planted done in stages ranging from 5 mL, 25 ml, 50 ml and 100 ml, to get the best colonies. Planting is done evenly on all the surface of the plate in order. LB plates that were then incubated at 37°C overnight. Results of bacterial growth in the form of colonies of blue and white, then recombinant colonies (white) were analyzed further.

*Analysis of recombinant pUC19.* Single colonies were brought pUC19 recombinant cultured into 25 ml LB medium containing ampicillin, and incubated at 37°C overnight with agitation speed of 200 rpm. Plasmid isolation is done in the same manner as in the preparation of plasmid pUC19. Four microliters of recombinant pUC19 with a concentration of 1 µg/ml, coupled with with 2 ml of 10X *Eco*RI buffer, 4 ml *Eco*RI and nuclease free water so that the total volume of the final 20 ml and mixed well. The reaction mixture was incubated at 37°C for 2 hours. Results of cutting reaction electrophoresed on a 1% agarose gel at 100 Volts.The addition of the adapter *Eco*R I use Riboclone®*Eco*R I Adaptor Ligation System. A total of 2.5 µl DNA (100 ng/µl) added 3 µl T4 DNA ligase buffer, 3 µl BSA (1 mg/ml), 1 µl *Eco*R I adapter, 2.5 U T4 DNA ligase and nuclease free water to a final volume of 30 µl. The reaction mixture was mixed well and incubated at 15$^{o}$C temperature overnight. To stop the reaction, the reaction mixture was incubated at 70$^{o}$C for 10 minutes.

### 2.11 Total Protein Isolation

A total of 30 ml culture of recombinant bacteria carrying pUC19 in LB media supplemented with ampicillin, centrifuged at 3000 rpm at a temperature of 4$^{o}$C for 10 minutes. The pellet was washed with PBSI three times by centrifugation at 3000 rpm at temperature of 4$^{o}$C for 10 minutes. Pellet diluted with 1 ml PBSI and broken by sonication for 30 seconds 5 times, at intervals of 1 minute. Supernatant obtained by centrifugation of 3500 rpm at temperature of 4$^{o}$C for 10 minutes. Samples were obtained used for immunoblotting after electrophoresis on SDS-PAGE.

### 2.12 Immunoblotting

Basic bloter moistened with bloting buffer, and coated stacked with Whatman paper that has been moistened with bloting buffer. Nitrocellulose membrane that has been moistened with bloting buffer placed on Whatman paper stack. SDS gel electrophoresis results placed on nitrocellulose membrane, and labeled with a 20-gauge needle as a sample layout orientation. Gel was closed as much as 4 papers Whatman layer that has been moistened with bloting buffer. Bloter closed with a cover bloter. Transfer of proteins performed on current red indicates the number 500, for 1-2 hours nitrocellulose membrane was removed and washed with TTBS 0.5%, for 30 minutes. Niroselulose membrane was blocked with 1% BSA, and incubated for 1 h with agitation at room temperature. Mouse monoclonal antibodies against protein membrane of *Toxoplasma gondii* local isolates (1: 500) was added and incubated for 1 h with agitation at room temperature. Washing performed 5 times with TTBS 0.05%, respectively washing for 10 minutes with agitation. Nitrocellulose membrane added with goat antimouse IgG alkaline phosphatase conjugate (1: 4000), and incubated for 1 hour at room temperature with agitation. Washing is done 5 times with 0.05% TTBS, each wash for 10 minutes with agitation. Nitrocellulose membrane coupled with a substrate solution containing NBT and BCIP in the dark room. The reaction was stopped by entering a nitrocellulose membrane in distilled water when it happens the formation of color. Nitroseluluse membrane was dried at room temperature and analyzed.

## 3. RESULTS AND DISCUSSION

The results of transformation use the pUC19 vector by electroporation, two clones of recombinant obtained as shown in Figure 1.

**Figure 1. Result of pUC19 transformation in *E. coli* DH5a by electroporation (White colony is recombinant, blue colony is no recombinant)**

pUC19 plasmid is one that is often used in genetic engineering. This plasmid has a size of about 2,686 kb, with the restriction to various endonuclease restriction, and the genes that encode markers of ampicillin resistance. The genes express the enzyme β-lactamase to the outside of the host cell. The enzyme catalyzes the hydrolysis reaction of the β-lactam ring causing bacteria transformed with pUC19 become resistant to ampicillin. Its fragments in *E. coli* lacZ genes that produce β-

galactosidase, the pUC19 can be identified by means of screening hystochemical or alpha complementation[15]. Genetically pUC19 lacZ operon having the nucleotide sequence derived from *E. coli*. LacZ operon into 14 first amino origin of β-galactosidase enzyme. If β-galactosidase is active it will hydrolyze a substrate X-gal and chromogenic dye is released to form blue colonies. Insertion of DNA in pUC19 polycloning site will inactivate β-galactosidase and eliminates the ability to complement on bacteria which carry recombinant pUC19 will form white colonies due to not being able to hydrolyze a substrate due to the formation of β-galactosidase enzyme active[15].

Low number of recombinant due to several factors such as the amount of cDNA that is too little. In just a few percent cDNA synthesis mRNA is synthesized into cDNA. Comparison between the cDNA plasmid that is not balanced will affect the outcome of the transformation, as well as some other factors in the transformation process.

From the results obtained recombinant bacterial multiplication recombinant pUC19 which can be seen in Figure 2.

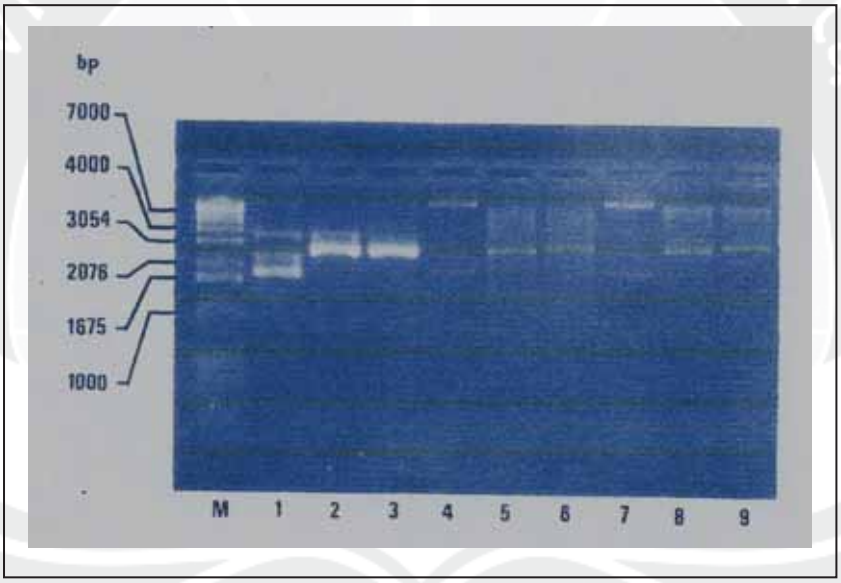


**Figure 2. Results of electrophoresis of pUC19 recombinant with cutting endonucleases restriction of *Eco*RI and *Hind*III**

(M, marker; 1, pUC19; 2, *Eco*RI cut pUC19; 3, pUC19 cut with *Eco*RI; 4, pUC19 recombinant 1; 5, pUC19 recombinant 1 cut with *Eco*RI; 6, pUC19 recombinant 1 cut with *Hind*III; 7, pUC19 recombinant 2; 8, pUC19 recombinant 2 cut with *Eco*RI; 9, pUC19 recombinant 2 cut with *Hind*III)

After electrophoresed on 1% agarose gel, the recombinant plasmid 1 and recombinant 2 shows the same banding pattern, but greater than the control pUC19. The big difference plasmid ribbon before being cut with restriction endonucleases enzyme, can be clarified with the recombinant plasmid cutting results with certain restriction endonucleases. Insertion of cDNA done on the restriction *Eco*RI know, so expect to cutting with the same enzymes obtained a piece or some pieces on fragment inserts. Results of electrophoresis on agarose gel 1% is not visible any fragments cutting inserts. The possibility of invisibility of long pieces of DNA fragments as small insertions, or DNA cut up into small fragments on a place known

by *Eco*RI, because the cloned DNA fragment contains the base sequence is unknown constituent. To prove the existence of inserts, used cutting with restriction endonucleases *Hind*III which recognize one specific side of the polycloning site on pUC19. Results showed that the obtained ribbon cutting linear larger than the control pUC19, thus allegedly carrying two recombinants cDNA having the same length, but not necessarily have the same base sequences.

Results recombinant protein immunoblotting 1 and 2 with several monoclonal antibodies can be seen in Figures 3 and 4.

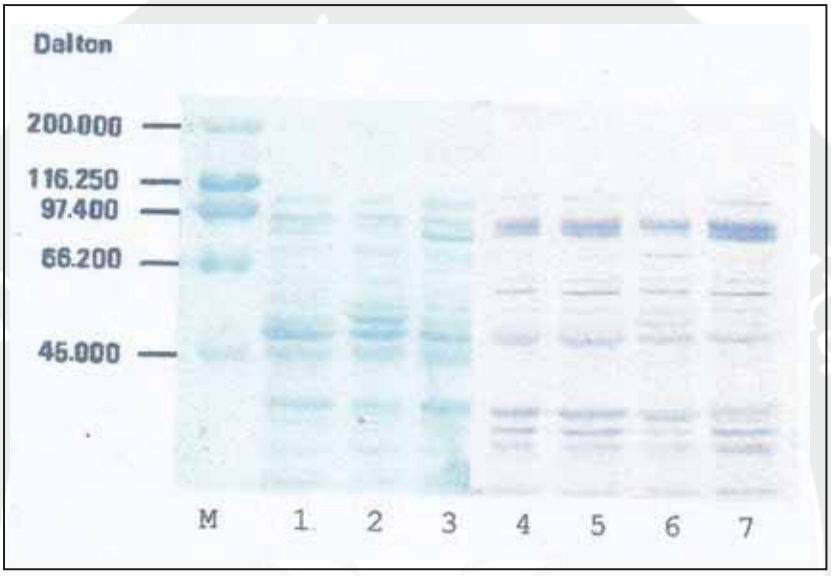


**Figure 3. Result of SDS-PAGE and Immunoblotting of recombinant 1 proteins**
(M, 1,2,3 Amidoblack staining; 3,5,6,7 the addition of monoclonal antibodies P55,1F6, D1, D9; M, protein marker; 1,4 protein of *E. coli* DH5α; 2,5 protein of *E. coli* DH5α with pUC19; 3,6,7 protein of *E. coli* DH5α with pUC19 recombinant).

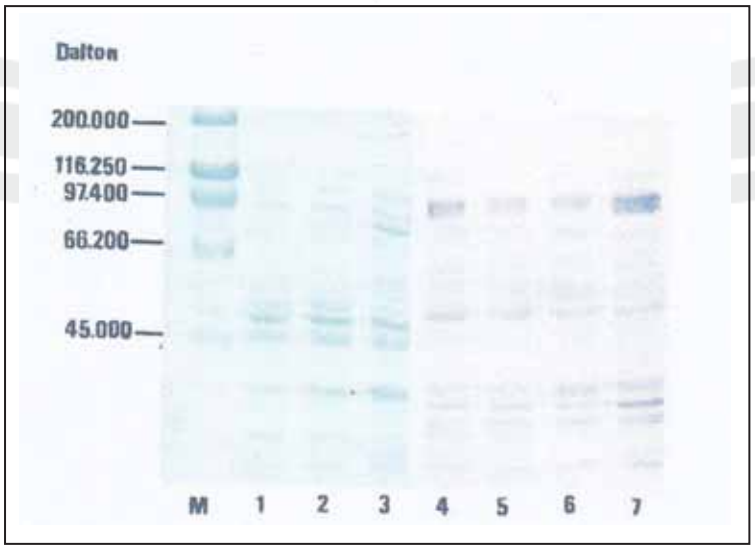


**Figure 4. Result of SDS-PAGE and Immunoblotting of recombinant 2 proteins**
(M, 1,2,3 Amidoblack staining; 3,5,6,7 the addition of monoclonal antibodies P55,1F6, D1, D9; M, protein marker; 1,4 protein of *E. coli* DH5α; 2,5 protein of *E. coli* DH5α with pUC19; 3,6,7 protein of *E. coli* DH5α with pUC19 recombinant)

Both recombinant proteins produced after reacted with monoclonal antibodies showed several bands with variations of a few bands in accordance with the monoclonal antibody used. These bands are also found in the control of bacterial proteins that carry pUC19 and who do not carry pUC19. This reaction occurs because monoclonal antibodies recognize specific epitopes on proteins. A minimal epitope consisting of amino acids making up 4-6, so there is the possibility of being used for screening antibodies recognize similar epitopes on proteins from bacteria. Monoclonal antibodies are antibodies produced by one type of cells (B lymphocytes) which are specific and homogeneous in nature, class, affinity and specificity, being a polyclonal antibody is an antibody that is heterogeneous who knows some epitope of an antigen.

The amino acid sequence of a protein in a species or other species in certain parts of the same possibilities. The existence of common epitopes on the protein will be known monoclonal antibodies that recognize the same epitope. [16]SAG1 and SRS2 on *Toxoplasma gondii* has a sequence that is similar to the NCP 29 and NCP 35 in *Neospora caninum*. Both protein in *Toxoplasma gondii* and *Neospora caninum* both have 12 amino acid cysteine residues. The use of monoclonal antibodies specific for clone selection purposes must have a high specification against epitopes on the protein expected but on the other epitopes. Another constraint that the foreign DNA proteins expressed in *E. coli* has a specific conformation or less different from the proteins that directly expressed by *Toxoplasma gondii* tachyzoite. Protein conformational epitopes will determine if the conformational change will lead to changes in the epitope of a protein that monoclonal antibodies do not recognize the epitope. [17]Antibodies against P30 P30 only recognize native nonreduced expressed *E. coli*. One of the obstacles the use of the expression vector pUC19 was not issued cell protein expression in sufficient quantities, and screening of bacterial colonies can not be done, so as to obtain the expected protein must break up the cells. Screening cDNA clones that carry *Toxoplasma gondii* monoclonal antibodies that are not specific to membrane protein of *Toxoplasma gondii* can not detect clones were expected. Another alternative to determine the clones which carry cDNA encoding a membrane protein of *Toxoplasma gondii* is using a vector that can express the protein out of the host cell and the antibody used for the screening does not react with the host cell expressed proteins.

## 4. CONCLUSION

Cloning of the gene encoding a membrane protein takizoit *T. gondii* using pUC19 in *E. coli* DH5a host got two recombinants.

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